

**A Breeding Method for Genetic Recombination for *Galinaceae*****Hybrids****Description**

[0001] The invention is directed to a breeding method for genetic recombination for *Galinaceae* hybrids, specially in the production of eggs for consumption.

[0002] The classical system of selection for the breeding of the existing breeds and lines of layers consists in the reproductive isolation of the valuable genotypes and their wide use for reproduction, concomitantly with the removal of the undesired genotypes.

[0003] However, a limit of the classical system of breeding is that the use of these breeds and lines in the industrial system resulted in attaining the peak selection performance (the genetic plateau), namely a production of around 220 eggs per layer.

[0004] This is due to the system of selection, which is based only on the active gene interactions, which increases the frequency of homozygote within the populations, homozygous trait compensated by other epistatic interactions, which maintained them in a genetic balance.

[0005] The increase of homozygote frequency beyond the limits of the genetic balance, in order to improve the laying percentage, leads to

the depression of the consanguinity and the onset of a genetic drift. Therefore, going beyond the selection plateau cannot be done only by the classical methods of selection applied on the layers stock.

[0006] Another problem insufficiently investigated, and therefore not yet clarified, concerns the genetic mechanism and the biochemistry of sex inheritance in poultry. Because the sex determination in poultry is a complex process, the identification of sex specific markers is of particular importance. Particularly useful are the markers, that can be applied to the identification of the sex in immature birds, before the occurrence of the specific morphological traits (secondary sexual characters such as the comb and the wattles). The early identification of sex in immature birds is of special importance to poultry breeding because such early identification allows the removal from reproduction and subsequent growth of the undesired genotypes (hybrid males). In accordance with this aspect, the procedure used in the invention aims to identify and screen the hybrid chicks according to their sex, based on the down color used as phenotypic marker, followed by a program of directed crossing aiming to produce genetic recombinants with good morphological and productive performance.

[0007] The technical issue of the invention consists of the induction of genetic recombination in the F1 generation, followed by progeny screening by sex as the result of sex genes interaction, using the juvenile feathers colour as phenotypic marker.

[0008] The procedure of genetic recombination for *Galinaceae* breeding, according to the invention, consists in the fact that, after the selection of parents, originating from pure, homozygous lines for the transmission of feather color, a red Rhode Island male is crossed with a barred Marans female, resulting in the F1 generation (figure 1).

[0009] The hybrid F1 progeny is sexed according to the color of feathers at one day old. When the progeny reaches the sexual maturity the heterozygous males and females are crossed, producing the F2 generation, which is also screened by phenotype categories, according to the color of feathers.

[0010] The progeny resulting from a homozygous recessive (bb) red Rhode Island male crossed with a homozygous dominant (BB) Marans female, genetically assessed when day-old, consists of 50% heterozygous males (Bb), with black juvenile feathers on the body and a white spot on the head, and 50% heterozygous females (bB) with black juvenile feathers on the body and head.

[0011] The F2 progeny resulting from a heterozygous male (Bb) crossed with a heterozygous female (bB) both from the F1 generation, genetically assessed when day-old, consists of 49.4% mixture of homozygous (BB) and heterozygous (Bb) females and males with black juvenile feathers on the body and a white spot on the head, 25.1% heterozygous (bB) females and males with black juvenile feathers on

the body and head and 25.5% recessive homozygous (bb) females and males with red juvenile feathers on the body and head.

[0012] The F2 offspring, assessed genetically at 18 weeks old, consists of 24.7% barred homozygous (BB) females and males with barred feathers, 25.1% reddish-black heterozygous (bB) females and males with black feathers on the body and reddish-black feathers on the neck and head, 25.5% gold-homozygous (bb) females and males with red feathers and 24.7% barred heterozygous (Bb) females and males.

[0013] The phenotypic distribution of the males in the last group showed 71.8% of the males having fully barred feathers and 28.2% of the males having barred feathers on the body and red feathers on the neck and head. The females of the same group were 100% barred.

[0014] Crossing the homozygous recessive (bb) red Rhode Island male with a homozygous dominant (BB) Marans female yielded in F1 generation 50% heterozygous females (bB) with black juvenile feathers on the body and reddish-black feathers on the neck and head, a combination of color that is different both from the red feathers of the recessive homozygous (bb) male parent and from the barred feathers of the heterozygous (Bb) males from the F1 generation. This feature is due to the dominant sex gene (SDW), located in chromosome W of the heterozygous (bB) females, that plays an epistatic action on the barred gene, which allows day-old recombinants sexing by the color of their juvenile feathers, while in relation with the recessive allele (sdw) located

in a homologous area in chromosome Z, it forms the heterozygous genotype (SDW sdw) of female sex; the recessive sex gene (sdw) in homozygous state forms the recessive homozygous genotype (sdwsdw) of male sex. Of the 50% barred heterozygous males (Bb), which resulted in F1 progeny, 71.8% had fully barred feathers and 28.2% had barred feathers on the body and red feathers on the neck and head.

[0015] The advantages of the invention are:

- highlighting the genetic mechanism of feather color inheritance;
- using the color of feather as a phenotypic marker for sexing day-old chicks;
- using the color of feather as a marker in highlighting the epistatic effect of the dominant sex gene on the barred gene, both located on chromosome W;
- increasing the morphological and productive performance compared to other hybrids for egg production.

[0016] The following is an exemplary procedure, according to the invention; figure 1 shows the diagram of the genetic recombinant production, while figure 2 shows a color photo of the F1 heterozygous female.

[0017] A total of 3275 day-old chicks hatched in the F1 generation, in two batches, from the eggs resulted by crossing a red Rhode Island

male with a barred Marans female. A number of 2633 young individuals were developed, macroscopically examined for the color of their feathers at the age of 18 weeks.

[0018] By crossing the F1 generation offspring (heterozygous males with heterozygous females), a total of 2440 hybrid day-old chicks were produced in F2 generation, after two hatching sessions, and 2294 chicks were examined macroscopically for the color of their feathers at the 18 weeks age.

[0019] The procedure according to the invention includes the following steps:

Step 1: Selection of the parents originating from pure lines, homozygous for feather color inheritance.

Thus:

- a) The male parent, phenotypic with red feathers, and genotypic recessive homozygous (bb) for the gold gene (b);
- b) The female parent, phenotypic it has barred feathers; while genotypic it is dominant homozygous (BB) for the barred gene (B);

Step 2: Crossing the recessive homozygous (bb), red Rhode Island males with dominant homozygous (BB) barred Marans females and production of the F1 generation of hybrids displaying two categories of phenotypes, one for each sex (figure 1).

[0020] Sex screening of day-old hybrids according to the color of the juvenile feathers is done as follows:

- The hybrid males have black juvenile feathers with a white spot of variable size on the head; genotypic they are heterozygous (Bb);
- the hybrid females have black juvenile feathers on the body and head; genotypic they are heterozygous (bB).

[0021] Characteristic to the inheritance of the color of the juvenile feathers to the day-old hybrid chicks is that the male parent (ZZ) transmitted the gold gene located in chromosome Z to both sexes of F1 generation.

[0022] In the female parent (ZW), the barred gene is located both in chromosome Z and in chromosome W. The barred gene is transmitted together with chromosome Z only to the heterozygous males and through chromosome W only to the heterozygous females.

[0023] The first observations on the hybrid combination are obtained after day-old chick sexing, when the heterozygous males are eliminated because they have no economic importance, while the heterozygous females are taken to specially fitted areas for growth and exploitation for eggs for consumption.

[0024] Monitoring the feather color of the hybrid males showed that at 18 weeks age, 71.8% of them show barred feathers, while 28.2% have barred feathers on the body and red feathers on the neck and head, while all heterozygous female had black feathers on the body and reddish-black feathers on the neck and head.

[0025] The feather color of the heterozygous female is different from the red colour of the male parents. Thus, obvious differences were observed between the feather color of the male parent and the heterozygous female produced in F1 generation, which shows that the mechanism of hemizygosity does not work at least in the case of feather color inheritance. These differences are explained by the presence of the gold gene (b) in chromosome Z and of the barred gene (B) in chromosome W in the heterozygous female, which has the heterozygous bB genotype.

[0026] The existence of a heterozygous genotype with a role in feather color inheritance in both sexes in the F1 generation, and of the two categories of phenotypes determined according to the feather color, which allow sexing, shows that in the genetic determinism of feather color of the heterozygous females, a third gene is acting besides the barred (B) and gold (b) genes. The action of the third gene determines the sex screening of the heterozygous females and males by the color of the juvenile feathers.

[0027] Hybrid chicks sexing by the color of the juvenile feathers are explained by the action played by the dominant sex gene, SDW, located in chromosome W on the barred gene.

[0028] The SDW gene plays two functions:

- It is a *dominant sex gene* in relation to its recessive allele, *sdw*, located in the homologous region in chromosome Z. The heterozygous

genotype SDWsdw determines the female sex, while the recessive homozygous genotype sdwsdw determines the male sex;

- It is functioning as an *epistatic gene*, which interacts with the barred gene also located in chromosome W. The non-allelic interaction (E) of the dominant sex gene (SDW) on the barred gene (B), overlaps the allelic interaction between the barred and gold genes, the latter one becoming non-functional and determining the appearance of only one phenotype category in the heterozygous females, which is black feathers on the body and reddish-black feathers on the neck and head. The presence of the dominant barred color transmitted by the female parent to the heterozygous (Bb) males in the F1 generation shows the action of the allelic interaction. The fact that the heterozygous females (bB) do not display any of the parental colors (dominant barred or recessive red) explains the lack of the dominant in the phenotypic expression. The heterozygous females have reddish-black feathers, different from the color of the parental feathers and from that of the heterozygous barred males. The color of the F1 heterozygous females feathers is due to the epistatic action of the dominant sex gene on the barred gene.

[0029] The reddish-black colour of the heterozygous females (figure 1) is determined by the action of the recessive gold gene located on the chromosome Z and of the hypostatic-barred gene located on the chromosome W.

[0030] The linked transmission of the genes involved in the feather color and sex determinism in poultry was revealed by the disagreement between the feather color of the male parent, which is fully red, and of the heterozygous females, which is black on the body and reddish-back on the neck and head.

[0031] In the proper crossings the male parent is recessive homozygous and the female parent is dominant homozygous and the feather color of the heterozygous females in F1 generation is a marker for the dominant sex gene.

[0032] **Step 3:** Crossing the F1 offspring between them and bearing of F2 generation.

Following the crossing of the heterozygous Bb males with the heterozygous bB females, three categories of phenotypes of day-old chicks are obtained, both sexes being equally represented:

- 49.4% a mixture of homozygous (BB) and heterozygous (Bb) females and males with juvenile black feathers on the body and a white spot on the head;
- 25.1% heterozygous (bB) females and males, with black juvenile feathers on the body and head;
- 25.5% homozygous (bb) females and males with red juvenile feathers on the body and head.

[0033] At 18 weeks of age, the progeny displayed a great variability of feather color, resulting in four categories of phenotypes in

males and three categories of phenotypes in females, in which both sexes are represented in equal proportions, as follows:

- 24.7% homozygous (BB), barred females and males with barred feathers;
- 24.7% heterozygous (Bb) barred females and males; 71.8% of the males had barred feathers and 28.2% had barred feathers on the body and red feathers on the neck and head, while all females showed barred feathers;
- 25.1% heterozygous (bB), reddish-black females and males with black feathers on the body and reddish-black feathers on the neck and head;
- 25.5% homozygous (bb), females and males with red feathers;

[0034] Equal shares between sexes were produced in F2 progeny for each category of genotype, their frequency being as follows: 24.7% dominant homozygous (BB), 25.5% recessive homozygous (bb) and 49.8% heterozygous (Bb/bB). Two groups of heterozygous were produced: 24.7% barred (Bb) and 25.1% reddish-black (bB).

Unlike the males, which display four categories of phenotypes, the females only have three categories of phenotypes because the mixture of barred homozygous (BB) and barred heterozygous (Bb) females has fully barred feathers on the body, neck and head and the assignment of these females in the two phenotypic categories described earlier was arbitrarily done.

[0035] The cause-effect relationship, namely the gene-color relationship, reveals the presence of the barred gene in chromosome W in the F1 heterozygous females and it is explained by the results of crossing the two heterozygous, namely Bb males x bB females, which yielded in the F2 generation four categories of phenotypes for the males and three categories of phenotypes for the females, compared to two categories of phenotypes obtained by T.H. Morgan (1919).

[0036] T.H. Morgan (1919) crossed Langshan males with barred Plymouth Rock females but he did not notice in this experiment the presence of the barred gene in chromosome W.

[0037] The development of the procedure of producing this hybrid, sexed by the down color, is based on the presence of the barred gene in chromosome W and F1 progeny sexing is explained by a new genetic mechanism, different from the mechanism of hemizygosity.

[0038] In F2 generation, the color of feathers is transmitted in both sexes for each of the categories of phenotypes that resulted, so the screening of the sexes of the recombinants according to this trait is only possible in the F1 generation.

[0039] The presence of the barred gene in chromosome W in F1 heterozygous females requires the review of the map of heterosomes, modified by F.B. Hutt in 1960. The necessity of reviewing this map relies on the results obtained and presented in this application for licence. The results have shown that in the heterosomes there is only

one polyallellic locus, for the genes playing a role in the genetic determinism of feather color, and not two loci, one for the gold and silver genes and the other for the barred and non-barred genes, as the current map of heterosomes shows.

[0040] It was observed that in order to draw the map of heterosomes and to determine only one polyallellic locus in which the genes coding for the color of feathers are located, must take into account that the heterozygous F1 reddish-black females have 100% black feathers on the body and reddish-black feathers on the neck and head, being determined genetically by the gold and barred genes, which form the heterozygous genotype bB . The fact that all females have the same phenotype is due to the linked transmission of the two traits, the color of feathers and the sex, concomitantly with overlapping the non-allelic interaction (E) of the dominant sex gene on the barred gene, with the allelic interaction between the barred and gold genes, the latter one becoming non-functional.

[0041] The existence of the non-allelic and allelic interaction, overlapping genetic phenomena occurring simultaneously, explains the black feathers on the body and the reddish-black feathers on the neck and head observed in the heterozygous (bB) females, which differ from the feathers of both the male parents (bb) and of the heterozygous males (Bb).

[0042] Unlike the heterozygous (bB) females, the allelic interaction between the barred and gold genes is present in the heterozygous (Bb) males. In the heterozygous males no epistatic action of the recessive sex gene (sdw) was noticed on the gold and barred genes located on the heterosome pair ZZ.

[0043] Investigation results show that the genes of the linkage group W have a particular manner of action within the poultry genome due to the epistatic action of the dominant sex gene (SDW).

[0044] According to the current map of heterosomes, the feather color of the heterozygous reddish-black females is determined by the action of the gold genes from the locus of silver and gold genes and of the barred gene from the locus of barred and non-barred genes. Genetically, between the gold and barred genes there should be a non-allelic interaction. Phenotypic, it was observed that not the epistasy, but rather the incomplete dominance leads to the barred feathers on the body and red feathers on the neck and head in 28.2% of the F1 hybrid males.

[0045] Investigation showed the existence of an allelic interaction between the barred and gold genes in the heterozygous (Bb) males. The allelic interaction is absent in the F1 heterozygous females. However, it was observed that phenotypic, the allelic interaction between the barred and gold genes in the heterozygous females is not functional due to the epistatic action of the dominant sex gene on the

barred gene. Due to this fact, the plumage color of the heterozygous (bB) females differ both from the color of the male parents (bb) and from the color of the F1 heterozygous males (Bb).

[0046] In order to obtain further information on the activity of the heterosomal barred and gold genes, parallel crossing between red Rhode Island males and barred Marans females, and between barred Marans males and red Rhode Island females was done. In F1 generation, 72% heterozygous males showed barred feathers on the body, neck and head, and 28% barred feathers on the body and red feathers on the neck and head. Unlike the heterozygous (Bb) males, the F1 heterozygous (Bb) females displayed barred feathers on the body, neck and head. However, a low number of hybrid females, less than 0.1%, displayed a reddish shadow on the neck and head, over the barred design of the feathers. The apparition of the red feathers on the neck and head in some heterozygous females reveals the presence of the gold gene on chromosome W and the epistatic action of the dominant sex gene on the gold gene.

[0047] The presence of the red color on the neck and head in 28% of the barred heterozygous (Bb) males and in 0.1% of the barred heterozygous (Bb) females reveals the presence of the dominant in the heterozygous males and the lack of the dominance in the barred heterozygous females due to the different frequency of the phenotypes representing the red color in the two sexes, caused by the epistatic

action of the dominant sex gene on the gold gene, which turns hypostatic.

[0048] By crossing the heterozygous (Bb) males with heterozygous (Bb) females from generation F1, three categories of genotypes resulted in generation F2, showing both the heterozygous (Bb) phenotype observed in the F1 heterozygous females and the presence of the gold gene in chromosome W.

[0049] Similar results with those expressed by the pair of genes barred – gold were also observed for the silver – gold pair of traits. Thus, crossing the homozygous (ss) red Rhode Island males with homozygous (SS) white Rhode Island females, the F1 homozygous (sS) females showed preponderantly red feathers, except for some white feathers accounted for by the presence of the silver gene in the chromosome W.

[0050] In F1 generation, 86.7% of the heterozygous (Ss) males had white feathers, the balance of 13.3% displaying white feathers with a few red feathers. Crossing heterozygous (Ss) males with heterozygous (sS) females, both parents from the F1 generation, the resulting F2 generation produced three categories of genotypes in which both sexes are equally represented.

[0051] In parallel with the crossing of (ss) red Rhode Island males with (SS) white Rhode Island females, homozygous (SS) white Rhode Island males were crossed with (ss) homozygous white Rhode Island

females. In F1 generation, the heterozygous (Ss) males have similar feather color with the heterozygous (Ss) males, which resulted from the previous crossing. The heterozygous (Ss) females had white feathers, except for 0.7% of them, which had a few red feathers scattered in the white feathers of the body. This phenotype was determined by the gold gene (s) acting under the epistatic effect of the dominant sex gene (SDW). In this case, both the gold (s) gene as well as the dominant sex gene (SDW) is located on chromosome W.

[0052] Another crossing used homozygous (bb) red Rhode Island males, the place of the homozygous (BB) barred Marans female being taken by bared homozygous (BB) Plymouth-Rock females. The results regarding the feathers colour obtained in generation F1 were similar with those obtained by crossing homozygous (bb) red Rhode Island males with homozygous (BB) barred Marans females.

[0053] The day-old chicks from the F1 generation displayed two phenotypes, one for each sex. The sexing of the day-old hybrid according to the color of feathers showed that:

- Phenotypic, the hybrids males had black juvenile feathers with a white spot of variable size on the head, while genotypic they were heterozygous (Bb);
- Phenotypic, the hybrids females had black juvenile feathers on the body and head, while genotypic they were heterozygous (bB).

[0054] After 18 weeks age, 72% of the heterozygous males (Bb) had barred feathers, while 28% of them had barred feathers on the body and red feathers on the neck and head. All heterozygous (bB) females had black feathers on the body and reddish-black feathers on the neck and head.

[0055] Crossing the gold (bb) males with barred (BB) females, the F1 heterozygous females displayed different feather color than the male parent, while crossing the barred (BB) males with gold (bb) females, the F1 heterozygous females displayed barred feathers on the body, neck and head, identical with the feathers of the male parent, except up to 0.1%, which display a red shadow on the neck and head.

[0056] We propose the introduction of the locus of the genes playing a role in the genetic determinism of the sexes in the map of heterosomes, as follows:

- The dominant sex gene, SDW, located on the chromosome W;
- The recessive sex gene, sdw, located on the chromosome Z.

[0057] The results obtained with the F1 hybrid females (figure 1) show that the dominant sex gene (SDW) is linked with the barred (B) gene, both genes being located on the chromosome W, while the recessive sex gene (sdw) is linked with the gold (b) gene, both located on the chromosome Z.

[0058] The linked transmission of the genes, which determine the color of the feathers and the sex, is characterized by the presence of

two loci in chromosome Z, with corresponding similar loci located on the chromosome W.

[0059] Considering the results of the investigations in the genetic determinism of the feathers colour and sex, we propose the “Gene theory of sexuality”, which is a continuation of the “Chromosomal theory of sex determination”.

[0060] The procedure of genetic recombination for breeding the *Galinaceae* hybrids specialized in the production of eggs for consumption, according to the invention, is different from the known hybrids by the fact that the inheritance of feather color is explained by a new gene mechanism, while the productive performances are improved compared to other laying hybrids that can be sexed by the down color.

[0061] The characteristics of the new hybrid show an increased number of eggs per production cycle, a lower feed intake for one kg egg mass and improved liveability. Figures 1 and 2 show the F1 hybrid female with the following characteristics: average size, elongated head, simple, vertical, teathed, bright red comb, large, vivid eyes, slightly bent, strong, yellowish-black beak, red wattles, averagely long neck properly covered by hackles the trunk frames within a round rectangle and has an horizontal position, long, horizontal back, wide breast, full, properly rounded, slightly bent towards the front, medium size wings, properly closed, displayed horizontally, strong feet dressed in feathers, black feathers on the body and reddish-black feathers on the neck and head.

[0062] The procedure of obtaining the new hybrid according to the invention relies on the cross between the red Rhode Island males with barred Marans females, as shown in figure 1.

[0063] The productive traits of this hybrid are as follows: 321 eggs per production cycle until 77 weeks age, average egg weight of 60.9 g and 64.9 g at 34 and 70 weeks, 20.1 kg egg mass with a feed conversion ratio of 2.25 kg feed per one kg of egg mass, average female weight of 2130 g when 34 weeks old, 50% laying percentage at 22 weeks, 96% and 95% viability of the young and adult females, respectively. This is a calm hybrid resistant to diseases.